# HABITAT ECOLOGY OF JUVENILE FRESHWATER MUSSELS (BIVALVIA: UNIONIDAE) IN A HEADWATER STREAM IN VIRGINIA

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#### ABSTRACT

The occurrence and distribution of juvenile freshwater mussels (ages 0-3 years) were assessed at a site on Big Moccasin Creek, southwestern Virginia, between January 1983 and March 1984. A circular bucket sampler (573 cm<sup>2</sup>) with a 130  $\mu$ m mesh bag net was used to collect 91 qualitative and quantitative samples from various habitats in the stream. A total of 92 juvenile mussels was collected; densities were greatest behind boulders and numbers were greatest in riffles and runs. Juveniles were decidedly clumped in distribution, and their occurrence was significantly correlated with the occurrence of fingernail clams. Most older juveniles (ages 2 and 3 years) occupied habitats similar to those inhabited by adults. The relatively high mean annual mortality of juveniles (approximately 44%), their low abundance, and the many age classes in each mussel population in Big Moccasin Creek appeared to indicate that low but relatively stable recruitment each year was sufficient to maintain a viable mussel assemblage in the stream.

The glochidia of freshwater mussels are obligate parasites on the gills or fins of fish, and if attachment to a suitable fish host occurs, the glochidia encyst, metamorphose, and excyst to begin their free-living stage as juveniles (sexually immature mussels) in the stream or lake bottom. Mortalities during this unique life cycle are believed to be greatest at two stages; unsuccessful attachment to the appropriate fish host and dropping from the fish into an unsuitable habitat. Contact with a fish host and the place of shedding young mussels from the host are largely due to chance, and only the juveniles that reach a favorable habitat survive (Howard, 1922). The presence of a byssus in juveniles of some species apparently serves for attachment to and stability in the substratum (Frierson, 1905). Although early investigators of mussel life histories recommended research on the juvenile stage (Coker et al., 1921), no such studies were conducted.

The location and habitat of juvenile mussels have been enigmas to malacologists, particularly in lotic systems. As in many taxa of aquatic fauna, conditions favorable for the juvenile stage can differ from those favorable for adults. Coker *et al.* (1921) noted that the study of habits and habitats of juveniles was difficult because the small mussels had rarely been collected. The juvenile shell up to 2 months of age is small (<1 mm long), transparent, and not calcareous (Howard, 1917); locating such specimens in a stream or river bottom is therefore difficult. Lefevre and Curtis (1912) reported that the juvenile period immediately following parasitism (lasting until approximately 20 mm in shell length) was the least known and least collected; later studies confirmed these early observations (Negus, 1966; Ahlstedt, 1979; Neves *et al.*, 1980).

With twenty-three species of freshwater mussels included in the federal list of endangered species, and designations of critical habitat in their respective recovery plans, the collection of new information on juvenile habitat and ecology is obviously critical. Casual observations and incidental data available on the juvenile stage are no longer sufficient to provide for the protection and enhancement of these and other declining populations of mussel species in the United States. Therefore, the objectives of this study were to locate juvenile

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mussels in a headwater stream and to describe the habitats used in this life history stage.

#### MATERIALS AND METHODS

Big Moccasin Creek (BMC) is a third-order stream flowing 88 km through Scott and Russell counties in southwestern Virginia and entering the North Fork Holston River near Weber City, Virginia. The study site (36º47'30''N, 83º11'50''W) is near the intersection of State Routes 676 and 677 (Owen's Farm) in Russell County. There the stream flows through open pasture; width and depth average 7.0 m and 0.2 m, respectively, during low flow conditions. Substratum composition is coarse particle sizes in runs and riffles, with sand and silt in pools. Water chemistry and temperature data for BMC were presented by Zale and Neves (1982a). This site was selected for study because the stream here is relatively small, easily accessible, and has a dense mussel assemblage consisting of seven mussel species: Medionidus conradicus (Lea), Villosa nebulosa (Conrad), V. vanuxemi (Lea), Pleurobema oviforme (Conrad), Fusconaia barnesiana (Lea), Lampsilis fasciola Rafinesque, and Alasmidonta viridis Rafinesque (Weaver, 1981; Neves and Zale, 1982; Zale and Neves, 1982b). The Asiatic clam, Corbicula fluminea (Müller, 1774), does not occur in upper BMC. Since a previous study in BMC indicated that mussels less than four years old were not sexually mature (Zale, 1980), we defined the juvenile stage as consisting of mussels of ages 0 to 3 years.

Three major habitat types (pool, run, and riffle) were identified for sampling. Pools were characterized by slow flow, greater water depth, and an overlying layer of silt on the stream bottom; runs had moderate current velocities, laminar flow, intermediate depths, and coarse substrata; and riffles had swift, turbulent flow, shallow depths, and coarse substrata. Two microhabitats were identified for sampling in these habitat types; the downstream side of boulders in the stream bed, and the area along stream banks.

In January and March 1983, initial qualitative samples of substratum were collected at the site to test the feasibility of sampling methods. An engine-driven centrifugal pump was tried but quickly became clogged by coarse substrata. Efforts to collect substratum samples with a vertical corer 5 cm in diameter were also unsuccessful because of the coarseness of subsurface substrata. All subsequent sampling for juvenile mussels was done with a circular (573 cm<sup>2</sup>) bucket sampler with a removable 130  $\mu$ m mesh nylon bag net attached to its downstream side. The sampler was pushed into the stream bottom, and all substratum was scraped into the net by hand and hand cultivator to the greatest depth possible. Each sample was emptied into a 13/ plastic bucket and fixed with 5% buffered formalin. We collected 16 preliminary samples of substratum from various habitats in the stream to determine whether juveniles could be located, and where subsequent sampling effort should be directed.

A systematic sampling design was used in each of the three major habitats. Three substratum samples were taken along transects in each habitat on 6 May, 7 June, 14 July, 12 September, and 28 October 1983. A total of 45 samples (3 samples from five transects in each habitat) were collected. Three samples from each microhabitat (behind boulders, along banks) also were collected on the following dates: 12 September and 17 December 1983; and 30 January, 5 March, and 23 March 1984. Because core sampling was not possible, we stratified microhabitat samples by depth. The upper layer of loose substratum was collected, and then using a hand cultivator to loosen the lower layer, as much of the remaining substratum as possible was removed separately. Sampling in BMC was limited to depths of about 15 cm because the deeper substratum was hardpan. Each layer was preserved and stored for later examination. Measurements taken concurrently with each substratum sample included water depth, and surface and bottom water velocity (with a pigmy current meter).

In the laboratory, each of the 75 quantitative samples was washed through a series of three U.S. Standard Sieves (6.5 mm, 2.0 mm, 125  $\mu$ m), sorted, and classified according to a modified Wentworth scale as follows (Hynes, 1970): cobble, 64-256 mm; pebble, 6-63 mm; gravel, 2-5 mm; sand, 0.06-1 mm; and silt, <0.06 mm. Cobble and pebble fractions of substratum samples were visually inspected for juveniles, and gravel and sand fractions were examined under a dissecting microscope at 12X magnification. Previous studies showed that no juveniles passed through the 125  $\mu$ m sieve (Zale and Neves, 1982b); consequently, the silt fraction was not inspected. Processing of each sample required 1 to 5 days, depending on the quantity and composition of substratum.

All juvenile mussels and fingernail clams (Sphaeriidae) were removed, counted, and placed in vials of 10% buffered formalin. Adult mussels in each sample were identified and counted. Cobble and pebble substratum fractions were airdried; gravel, sand, and silt components were oven-dried at 100°C for 48 hrs. Each dried fraction was weighed on a triple beam balance to determine particle size composition, by weight, of each sample. Densities of juvenile mussels and sphaeriids were computed per sample and converted to numbers per square meter of substratum sampled. Juveniles were aged in years by counting growth rings on the external surface of valves and tentatively identified to genus by comparing the umbonal beak sculpture with that on the shells of adult mussels from the study site. Shell lengths and widths of juveniles were measured with vernier calipers or with an ocular micrometer under a dissecting microscope.

Kruskal-Wallis one-way analysis of variance was used to determine whether mollusc abundance differed significantly among habitat types. Two dependent variables, juvenile mussel and sphaeriid densities, were tested against water depth, surface and bottom current velocity, and percent cobble, pebble, gravel, sand, and silt. Spearman rank correlations were used to determine relationships between densities of juvenile mussels, fingernail clams, and measured physical variables (Zar, 1974).

To obtain an estimate of the number of juvenile mussels in this 100 m section of BMC, the site was physically surveyed by transects, mapped, and categorized into the five habitat types on the basis of stream bottom areas measured. Using the area-density method, we multiplied mean densities of juveniles in each habitat type by total area of that type to estimate abundance (Everhart *et al.*, 1975). Numbers per habitat type were summed to estimate total number of juveniles. A survival estimate of juveniles of all species combined was calculated using the relative abundance of each juvenile cohort (ages 0-3 years) in the 75 quantitative samples, according to the Robson and Chapman method (Ricker, 1975).

## RESULTS

We collected 17 juvenile mussels in the 16 preliminary samples. Sphaeriids were common in all samples but juvenile mussels occurred only in samples from riffles and runs. Later quantitative samples collected from March 1983 to March 1984 differed in the occurrence of juveniles among habitat types, although some were taken in all habitats sampled. Totals of 75 juvenile and 36 adult mussels were collected in the 75 quantitative samples taken on the nine sampling dates (Table 1). Juveniles were present in only 30 of the 75 samples and were clumped in distribution (Fig. 1). For example, 18 of the juveniles taken behind boulders were in 2 of the 15 samples from this microhabitat.

Sphaeriids were relatively common in all samples and occurred, in order of decreasing abundance, in the pool, runs, behind boulders, along banks, and riffle habitats. Three species of fingernail clams were identified: *Pisidium compressum* Prime, *P. casertanum* (Poli), and *Sphaerium striatinum* (Lamarck). A clumped distribution of sphaeriids was also evident but no distributional analysis by species among habitat types was attempted.

Table 1. Number, age group, and location of mussels collected in75 quantitative samples from Big Moccasin Creek on nine samplingdates, May 1983 to March 1984.

HABITAT JUVENILE AGE GROUPS (yrs) ADULTS							TOTAL
	0	1	2	3	Total Juveniles	≥4	
Pool	0	4	3	1	8	0	8
Run	4	3	3	1	12	7	19
Riffle	5	6	3	3	17	13	30
Boulder	15	6	7	7	35	8	43
Bank	1	1	0	1	3	8	11
TOTAL	25	20	16	14	75	36	111

Because juvenile mussels and sphaeriids showed a clustered distribution, and sampling covered only a small fraction of total habitat, our computed estimates of bivalve densities are considered to be only rough approximations (Table 2). Densities (no./m<sup>2</sup>) based on sampling results ranged from 0 to 52 juveniles in riffles, pools, and runs; 0 to 17 along stream banks; and 0 to 175 behind boulders. The wide ranges reflect the apparently clustered distribution of this life history stage.

Of the 92 juvenile mussels collected in qualitative and quantitative samples from BMC, 69 were less than 15 mm long (range 0.8 - 30.3 mm). Identifications were as follows: 50 Villosa spp., 34 Medionidus conradicus and 8 Fusconaia barnesiana or Pleurobema oviforme. Four age classes (0-3) were identified, with slightly more specimens in age classes 0 and 1 (Table 3). Mean lengths of juveniles ranged from 2.7 mm for age 0 to 23.2 mm for age 3. Age 0 individuals were most commonly collected behind boulders and were absent

POOL



Fig. 1. Location of samples, and the number and location of juvenile freshwater mussels collected at the study site in Big Moccasin Creek. Numbers indicate number of juveniles collected at that location; ● represents sample locations without juveniles; S and B identify microhabitat samples along streambanks and behind boulders, respectively.

 Table 2. Number and weighed mean densities (no./m²) of juvenile

 mussels and fingernail clams in 75 quantitative samples from Big

 Moccasin Creek on nine sampling dates, May 1983 to March 1984.

HABITAT	JUVEN	ILE MUSSELS	SPHAERIIDS		
	No.	Density	No.	Density	
Pool	8	9.3	1046	1218	
Run	12	15.1	616	717	
Riffle	17	25.6	162	189	
Boulder	35	39.6	570	664	
Bank	3	2.3	478	557	

 Table 3. Cohorts and sizes of all juvenile mussels collected in Big

 Moccasin Creek, May 1983 to March 1984.

AGE	NO.	SHELL	LENGTH	(mm)	SHEL	L WIDTH	(mm)
		mean	range	SD	mean	range	SD
0	27	2.7	0.8-5.0	1.22	1.8	0.6-3.4	0.80
1	25	6.4	2.2-11.0	2.32	3.6	1.6-5.4	1.05
2	20	13.6	4.5-21.2	3.82	7.8	2.9-12.9	2.29
3	20	23.2	11.2-30.3	5.68	12.9	5.7-17.2	2.71

in the pool samples. Adult mussels, which occurred most frequently in riffle samples, were also absent in the pool (Table 1); however, some adults were seen in pools during low flow conditions. A relatively wide size range within cohorts, most evident in ages 2 and 3, was attributed to differences in species and growth rates. One specimen 25.7 mm long (age 3) was gravid but was nevertheless included in the juvenile category because eight larger juveniles (> 25 mm shell length) were immature. Mean annual survival for juveniles, as determined by the Robson-Chapman method, was 56% for ages 0 to 3 years. This estimate of juvenile mortality (44.56 per year) excludes the high mortality reported to occur within a few days after mussels drop from the fish host.

Occurrence of juvenile mussels behind boulders in the stream was most often in the upper stratum of samples (0-8 cm deep). Of the 26 juveniles collected in these quantitative samples, 20 were in the surface layer.

Differences in densities of juveniles among the five habitat types, statistically analyzed with a Kruskal-Wallis test,

were significant (p = 0.01). Because of the large number of samples that contained no juveniles (45 of 75), a chi-square contingency test was used to corroborate results of the Kruskal-Wallis test. Chi-square analysis confirmed that juvenile densities were significantly different among habitat types ( $\chi^2$  = 44.3; p < 0.001). Multiple comparison tests made with these mean density data indicated that the density of juveniles behind boulders was significantly greater than that in pool habitat (p = 0.009) or along banks (p = 0.001), and significantly lower along stream banks than in riffles or runs (p = 0.02).

Kruskal-Wallis tests (p = 0.05) used to compare bivalve densities and environmental variables also revealed significant associations (Table 4). Multiple comparison tests between juvenile mussel abundance and the five habitat types indicated significant differences between the following: pool and boulder, run and bank, riffle and bank, and boulder and bank. These four paired comparisons also differed significantly in bottom and surface current velocities, indicating that the occurrence of juvenile mussels was correlated with water velocity in these habitats. Comparable tests with fingernail clam data showed significant differences between pool and riffle, run and riffle, and riffle and boulder habitats. No consistent trends between bivalve densities and substratum type were evident.

Spearman rank correlation tests between juvenile mussel densities and other measured variables indicated a significant association only with sphaeriid densities (p = 0.05). Areas in the stream with the most juvenile mussels also had the most sphaeriids. These correlation tests were influenced to a considerable degree by the relatively small numbers of juveniles and the many samples from all habitats that included no juveniles. Because of these two factors, sensitivity of the statistical tests is considered low.

As judged by the density of juvenile mussels and fingernail clams in each habitat and the total areas of those habitats, approximately 11,000 juvenile mussels and 582,000 fingernail clams occurred within our 100 m section of BMC (Table 5). Although juveniles were in greatest density behind boulders in riffles and runs, this habitat type composed only 0.9% of the stream bottom and supported less than 3% of

 
 Table 4. Summary of habitat data, mean and range (in parentheses), collected with quantitative samples from Big Moccasin Creek, May 1983 - March 1984.

HABITAT	WATER DEPTH	VELOCITY (cm/s)		SUBSTRATUM (%)					
	(cm)	Surface	Bottom	Cobble	Pebble	Gravel	Sand	Silt	
Pool	25	5	4	6	53	20	21	<1	
	(14-40)	(0-36)	(0-17)	(0-23)	(45-63)	(11-31)	(9-28)	(0-2)	
Run	22	20	12	31	49	11	9	<1	
	(12-31)	(3-53)	(0-30)	(4-61)	(31-64)	(2-24)	(1-13)	(0-1)	
Riffle	19	36	33	33	50	10	7	<1	
	(7-32)	(6-78)	(6-78)	(12-49)	(39-62)	(3-19)	(2-16)	(0-2)	
Boulder	24	32	32	34	43	12	11	< 1	
	(7-38)	(0-92)	(0-92)	(0-71)	(23-64)	(2-28)	(4-25)	(0-1)	
Bank	28	10	10	23	52	11	13	<1	
	(6-39)	(0-49)	(0-70)	(22-67)	(2-23)	(4-28)	(0-2)		

the total estimated juveniles present. A total of 8139 (75%) of the 10,830 juveniles at the site were in riffles and runs, which together accounted for roughly 55% of the stream bottom area. Juvenile densities were lowest along the stream banks and in pools, but the relatively large area of pool habitat (28,1%) accounted for 19% of the total juveniles.

**Table 5.** Estimates of juvenile mussel and fingernail clam abundance at the study site (100 m long) in Big Moccasin Creek, based on the area-density method.

HABITAT	AREA	PERCENT	MUS	SELS	CLAMS		
TYPE	(m²)	AREA	(no./m²)	Total	(no./m²)	Total	
Run	283	35.6	15.1	4273	717.1	202,939	
Riffle	151	19.0	25.6	3866	188.6	28,479	
Pool	224	28.1	9.3	2083	1217.7	272,765	
Boulder	8	0.9	39.6	309	663.6	5,309	
Bank	130	16.4	2.3	299	556.5	72,345	
TOTAL	796	100.0	-	10,830	-	581,837	

## DISCUSSION

The contagious distribution of juvenile mussels among habitats and samples within habitats in BMC accounted in part for the difficulty in locating juveniles, as described in earlier studies (Isely, 1911; Coker et al., 1921). Our results and those of previous studies in rivers concur in juvenile habitat description; namely, swift water with substrates of coarse gravel and boulder. Early investigators consistently reported the occurrence of a byssal thread on juvenile mussels, first observed after about 38 days (Isely, 1911; Howard, 1922). In Oklahoma rivers, Isley (1911) found juveniles attached to rocks and pebbles where water currents were swift. We observed few juveniles with a byssus, but because of the methods used to obtain and process substrate samples, byssal threads extruded by juveniles were probably broken.

The relatively high abundance of age 0 mussels behind boulders in riffles and runs has not been previously reported. The tendency of currents in streams to deposit finer particulate and organic matter in the eddies behind boulders, may account for their greater occurrence at these locations. Except for typically smaller particle sizes in the surface layer of substrate behind boulders, the overall composition of substratum down to roughly 15 cm was similar to that in other habitats. Since most of the juveniles were in the upper portion of substratum (0-8 cm), environmental conditions in this unconsolidated substratum were presumably suitable for young mussels.

The habitat for juvenile mussels in lotic systems differs from that reported for lakes. Juveniles of lake species have been collected primarily in sandy substrata (Coker et al., 1921; James, 1985). Ecological adaptations, even at the juvenile stage, can exist between lotic and lentic species, as well as among lotic species in headwater streams versus large rivers. Just as adults of many mussel species exhibit non-random distributions in response to environmental conditions, we suspect that subtle microhabitat preferences also occur among juveniles of at least some species. However, information on this early life stage is inadequate to enable us to judge whether the distribution of juveniles in BMC was due to differential survival among habitat types, habitat preference, or excystment of newly metamorphosed juveniles from host fish into those habitats.

Natural mortality appears to be high during the first year of life, since Howard (1922) reported a scarcity of young mussels even a few days after metamorphosis. Predators such as turbellarians and fishes take their toll, but the greatest natural mortality is believed to result from the mussels falling into unfavorable habitat or from the effects of spates on settled juveniles (Coker et al., 1921). Microhabitat preferences of stream fishes are well documented (Gorman and Karr, 1978; Gatz, 1979), and the following species serve as hosts for the dominent mussel species in BMC (Weaver, 1981; Zale and Neves, 1982b): smallmouth bass (Micropterus dolomieui Lacépède), rock bass (Ambloplites rupestris Rafinesque), banded sculpin [Cottus carolinae (Gill)], redline darter [Etheostoma rufilineatum (Cope)], fantail darter (E. flabellare Rafinesque), central stoneroller [Campostoma anomalum (Rafinesque)], river chub [Nocomis micropogon (Cope)], war paint shiner [Notropis coccogenis (Cope)], and whitetail shiner [N. galacturus (Cope)]. Since most of these species are considered to be riffle-dwellers, newly metamorphosed mussels would likely be dropped into riffles. The correlation between density of juveniles and water velocity tends to support this observation. Howard (1922) reported that young mussels, in suitable substratum and undisturbed, seemed to be relatively inactive. If these early observations are correct, the juveniles collected behind boulders and in riffles in BMC may remain there for several years before seeking habitat characteristic of adults of their respective species. Displacement of juvenile mussels by flooding undoubtedly occurs, and passive movements may account for shifts in the distribution of these young cohorts. Ecological and habitat requirements of the juvenile stage remain essentially unknown.

Our estimate of roughly 11,000 juvenile mussels at the study site can be compared with an estimate of adult mussels within a reach of BMC that included our 100 m site. Quadrat sampling of adult mussels in this reach provided an estimate of 50,580 adult mussels in 2700 m<sup>2</sup> of run and riffle habitats (Weaver, 1981). Assuming few adults in the pool habitat, this estimate of abundance suggests that roughly 11,000 adult mussels also occurred within our study site. The entire mussel assemblage in this 100 m section of stream therefore consisted of approximately 22,000 adults and juveniles. *Medionidus conradicus* was the most common species of the adults collected in quadrat samples (Zale and Neves, 1982a), but *Villosa nebulosa* and *V. vanuxemi* were tentatively identified as most abundant among the juveniles collected.

In a previous study of age class structure of the more common species in BMC, Zale (1980) calculated an adult mortality rate of 7 to 19% among ages 4 to 9 years. In the Thames River, Negus (1966) reported annual mortality rates of 5 to 12% for adult *Anodonta anatina* (Linné). It thus appears that mortality declines significantly after mussels reach sexual maturity. The large number of age classes in the mussel populations of BMC (Zale, 1980; Moyer, 1984), and the high mortality of juveniles and their relatively low abundance, all indicate that low but apparently continuous annual recruitment is sufficient to maintain a healthy mussel assemblage in BMC.

To obtain an alternate estimate of adult mussel abundance at the study site for comparison with the quadrat value of 10,715 adults, we used the best available data on population statistics. Previous investigations have calculated annual mortality rates of 5 to 19% for adult mussels (Negus, 1966; Zale, 1980), and maximum ages of the species in BMC between 22 and 56 yrs (Moyer, 1984). To compute a range for the number of mussels at the site, we used our estimate of ages 3 juveniles (2058) as the typical cohort size; used two mean annual mortality rates (10 and 15%) for cohorts of age 4 and older; and assumed a somewhat conservative maximum age of 22 yrs for all species. The number of individuals in each computed cohort (all species combined) was summed between ages 4 and 22 to provide a theoretical estimate of adult mussels at the site. Our estimate was 16,019 mussels, based on an adult mortality rate of 10%, and 11,132 mussels based on 15% annual mortality. The estimate of adults based on a mortality rate of 15% compares favorably with the initial estimate from previous quantitative sampling. Although several assumptions were made in using these population data and treating all species together, we believe that the admittedly rough estimates of mussel abundance for juveniles and adults provide a realistic assessment of the mussel assemblage at this site.

Our success in locating juvenile mussels in BMC is attributed to the reproductive success of apparently healthy populations and the meticulous procedure for processing samples to locate specimens. The juvenile stage is by no means abundant, and the contagious distribution of these early cohorts necessitates numerous samples, even in known habitat, to document their occurrence at specific locations in streams. Although the lack of juveniles (poor recruitment) in other studies has been attributed to sedimentation, pollution, or eutrophication (James, 1985), many of these previous failures to locate juveniles in streams and rivers can probably be attributed to insufficent or inefficient sampling.

The correlation between the abundance of juvenile mussels and that of fingernail clams, and the numerous habitats occupied by the invading Asiatic clam (Corbicula fluminea) in BMC and other streams are cause for concern. Although spatial competition between this exotic clam and adult freshwater mussels was postulated (Fuller and Imlay, 1976; Kraemer, 1979), we believe that the juvenile stage of mussels is probably most susceptible to competitive interactions for space or food with this species. The mode and efficiency of reproduction weigh heavily in favor of the Asiatic clam, and declines in mussel populations may go unrecognized for several years because of the difficulty in collecting younger cohorts. It appears therefore that documenting the presence of juvenile mussels in a mussel assemblage may be the only sure way of assessing the relative viability of those populations.

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